Reference: *Biol. Bull.* 172: 132–136. (February, 1987)

PHARMACOLOGICAL ACTIONS OF THE MARINE TOXINS CIGUATOXIN AND MAITOTOXIN ISOLATED FROM POISONOUS FISH

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Abstract

Ciguatoxin and maitotoxin isolated from poisonous fish and toxic dinoflagellates exhibited a powerful excitatory effect on smooth and cardiac muscle. The ciguatoxin-induced excitatory action is due to an increase in Na^+ permeability of tetrodotoxin-sensitive Na channels, while the maitotoxin-induced excitatory effect is caused by an increased Ca^{2+} permeability of the cell membrane.

Introduction

A wide range of pharmacologically useful substances has been isolated from marine organisms. Among them, marine toxins such as tetrodotoxin (TTX), saxitoxin and sea anemone toxins, have been extensively studied since they affect a specific physiological function (Catterall, 1980). The first of these toxins, TTX, was isolated from puffer fish. It specifically blocks voltage-sensitive Na channels (Narahashi, 1974). Thus, TTX has been an essential tool in elucidating neurally mediated physiological processes. Such neurotoxins as TTX and saxitoxin, which bind with high affinity to Na channels, have proved to be useful probes for purifying and identifying these channels (Catterall, 1984).

Ciguatera is a disease caused primarily by the ingestion of any of a variety of fish inhabiting tropical and subtropical seas. The clinical symptoms for ciguatera are prickling of the lips, tongue, and throat; numbness; nausea; vomiting; metallic taste; dryness of the mouth; abdominal cramps; and diarrhea. It is well known that the principal toxin in ciguatera fish is ciguatoxin (CTX), a hydrohobic compound (Hashimoto, 1979). CTX is a highly toxic substance; the 50% lethal dose in mice is 0.45 μg/kg when injected intraperitoneally (i.p.) (Tachibana, 1980). Recently, maitotoxin (MTX), a water-soluble substance, has been isolated from the viscera of a surgeonfish, *Ctenochaetus striatus,* and from the toxic dinoflagellate *Gambierdiscus toxicus;* it was found to be the most potent marine toxin known. The minimum lethal dose of MTX in mice is 0.17 μg/kg (i.p.) which is approximately fifty times more potent than that of TTX (Yasumoto, 1980). The chemical structure of MTX has been only partially determined although it is considered to be a non-peptidic compound with a high molecular weight (Yasumoto, 1980).

Materials and Methods

Mechanical response

Male guinea-pigs (250–350 g) and male rats (280–300 g) were used. The guinea-pig vas deferens and left atria were excised and mounted vertically in a 20 ml organ bath containing an Krebs-Ringer bicarbonate solution. The procedures for preparing the vas deferens (Ohizumi and Shibata, 1980) and the left atria (Kobayashi *et al.,* 1985a, b) were carried out as previously described. Cardiac myocytes were isolated from the rat using the method of Miyakoda and Nakamura (1982) and driven by a

TABLE I

Effect of CTX on the NE release from the guinea-pig vas deferens in the presence or absence of TTX or Ca^{++}

Treatment	Amount of NE released[a] ng/g tissue/30 min
None	39.3 ± 6.3
CTX (10^{-6} g/ml)	1225.0 ± 240.8
TTX (5×10^{-7} M)[b] + CTX (10^{-6} g/ml)	45.0 ± 9.7
Ca^{++}-free medium[b] + CTX (10^{-6} g/ml)	82.8 ± 11.9**

[a] Mean ± S.E. (n = 4).
[b] CTX was applied 15 min after treatment with TTX or incubation in Ca^{++}-free medium.
** Significantly different from the CTX-treated group, $P < .01$.

stimulator through a pair of platinum electrodes. Images of the cells were recorded either with a video recording system or with a high-speed movie camera.

Assay of norepinephrine (NE)

Preparation and incubation of the guinea-pig vas deferens were performed as described previously (Norton *et al.*, 1981). The determination of released endogenous NE was carried out as described by Ohizumi *et al.* (1983).

Tissue Na and Ca content

The Na (Ohizumi *et al.*, 1982) and Ca (Ohizumi *et al.*, 1983) concentrations in tissue were determined by the methods described earlier.

Na,K-ATPase assay

Na,K-ATPase was purified from the porcine brain. The enzyme reaction was carried out at 37°C, for 20 min as described previously (Ohizumi and Yasumoto, 1983), and inorganic phosphate was determined according to the method of Martin and Doty (1949).

Extraction and purification of CTX and MTX

CTX (Tachibana, 1980) and MTX (Yasumoto, 1980) were extracted and purified as described previously.

RESULTS AND DISCUSSION

Smooth muscle

CTX induced a dose-dependent contraction of the guinea-pig vas deferens at concentrations above 3×10^{-7} g/ml (Ohizumi *et al.*, 1981). The CTX-induced contraction of the vas deferens was inhibited by phentolamine (10^{-6} *M*), guanethidine (10^{-4} *M*), reserpine (2 mg/kg/day, twice), TTX (5×10^{-7} *M*), or cold storage for 7 days at 4°C, but was not affected by atropine (10^{-6} *M*) or mecamylamine (3×10^{-5} *M*). CTX ($1–6 \times 10^{-7}$ g/ml) caused a dose-dependent release of NE from the vas deferens, and the maximum response (1297.4 ng/g tissue) was obtained with a concentration of 6×10^{-6} g/ml. The CTX-induced release of NE was nearly abolished by TTX (Table I) or by cold storage. These data suggest that the CTX-induced contraction of the vas deferens results from an indirect action mediated through NE release from the adrenergic nerve terminals, and that CTX causes an increase in Na^+ permeability of the presynaptic membrane, which may play an important role in its releasing action.

The dose-contractile response curves for the action of NE and KCl on the vas deferens were shifted to the left in a parallel manner by CTX, indicating that CTX caused supersensitivity (Ohizumi *et al.*, 1982). The CTX-induced potentiation was abolished by TTX (5×10^{-7} *M*) or saxitoxin (5×10^{-7} *M*); it was inhibited by a Na^+-deficient medium, but was not affected by phentolamine (10^{-6} *M*). After treatment with reserpine (2 mg/kg/day, twice) or cold storage (4°C, for 7 days), CTX-induced release of NE was completely prevented. CTX still markedly potentiated the response to NE and KCl in the reserpinized preparation or the cold stored preparation. On the other hand, tissue Na content of the vas deferens was markedly increased by ouabain (10^{-5} *M*), but was not affected by CTX at concentrations between 1 and 5×10^{-7} g/ml. However, in the presence of ouabain (10^{-5} *M*), Na content was increased 30% or more by CTX (5×10^{-7} g/ml). This increasing effect of CTX on Na content was abolished by TTX (10^{-6} *M*) (Ohizumi *et al.*, 1982). These data suggest that CTX causes an increase in Na^+ permeability across the TTX-sensitive Na channels of smooth muscle cells, and this may play an important role in its mechanism of potentiation.

The responses of the guinea-pig vas deferens to MTX were different from those of CTX. MTX (10^{-9} to 3×10^{-8} g/ml) caused a dose-dependent slower contraction of the vas deferens (second phase) that followed the initial rapid phasic contraction (first phase) (Ohizumi *et al.*, 1983). The second component of the MTX-induced contraction was markedly inhibited by phentolamine (10^{-6} *M*) and reserpine (2 mg/kg/day, twice), whereas the first component remained unaffected. Both components were inhibited or abolished by verapamil (10^{-6} to 10^{-5} *M*) or Ca^{2+}-free medium, but were not affected by atropine (10^{-6} *M*), chlorpheniramine (10^{-6} *M*), or TTX (10^{-6} *M*). The tissue Ca content of the vas deferens was increased by MTX (10^{-9} to 3×10^{-8} g/ml) in a dose-dependent manner. Furthermore, MTX (10^{-9} to 3×10^{-8} g/ml) caused a dose-dependent release of NE from the vas deferens, which was inhibited or abolished by verapamil (3×10^{-6} and 10^{-5} *M*) or Ca^{2+}-free medium, but not by TTX (10^{-6} *M*). In Na^+-free medium, MTX still caused a marked increase in NE release from the tissue (Table II). It has been reported that MTX elicited Ca-dependent excitatory effects on neuronal cells (Takahashi *et al.*, 1982, 1983; Freedman *et al.*, 1984). These observations suggest that the major part of the first component of the MTX-induced contraction is the result of a direct action of MTX on the smooth muscle membrane, whereas the second component is primarily the result of indirect action mediated through the NE release from the adrenergic nerve terminals. The data also suggest that Ca^{2+}, but not Na^+ is indispensable for the action of MTX.

In the isolated guinea-pig and rat ventricle strips, MTX (10^{-10} to 4×10^{-9} g/ml) caused a dose-dependent inotropic effect. The MTX-induced inotropic effect was nearly abolished by Co^{2+} (2 m*M*) or verapamil (3×10^{-7} *M*), but was little affected by propranolol (10^{-6} *M*), reserpine (2 mg/kg, twice), or TTX (5×10^{-7} *M*) (Kobayashi *et al.*, 1985). The tissue Ca-content of guinea-pig left atria was increased by MTX (2–3 $\times 10^{-8}$ g/ml), and this increase was markedly inhibited by Co^{2+} (2 m*M*) or verapamil (10^{-5} *M*). In rat myocardial cells, MTX (10^{-10} to 10^{-9} g/ml) induced an increase in the degree and the rate of contraction and subsequent arrhythmogenic actions (Kobayashi *et al.*, 1985). These effects of MTX were antagonized by verapamil (10^{-6} *M*) and were completely inhibited by Ca^{2+}-free medium. When the concentration of external Ca^{2+} was increased to 2 m*M*, MTX (10^{-8} g/ml) made all the rod cells turn into arrhythmically moving cells and then into rod-shaped cells much faster than was the case in the control medium. These results suggest that the excitatory effect of MTX on heart muscle is caused by a direct action on the cardiac muscle membrane, due mainly to an increase in Ca^{2+} permeability, possibly through some Ca channels.

TABLE II

Effect of MTX on the NE release from the guinea-pig vas deferens in the presence or absence of TTX or verapamil

Solution Treatment	Amount of NE released[a] ng/g tissue/30 min
Normal solution	
None	22 ± 4
MTX (3×10^{-8} g/ml)	1350 ± 169
MTX (3×10^{-8} g/ml) + TTX (5×10^{-7} M)[b]	1397 ± 205
MTX (3×10^{-8} g/ml)	
+ verapamil (3×10^{-6} M)[b]	770 ± 81*
+ verapamil (10^{-5} M)[b]	548 ± 62*
Ca^{++}-free solution[c]	
None	18 ± 3
MTX (3×10^{-8} g/ml)	97 ± 11
Na^{+}-free solution[c]	
None	832 ± 54
MTX (3×10^{-8} g/ml)	2973 ± 134
MTX (3×10^{-8} g/ml) + verapamil (10^{-5} M)[b]	2010 ± 110*

[a] Mean ± S.E. (n = 6).

[b] TTX and verapamil were added 15 min before the application of MTX.

[c] Tissue were incubated for 15 min in Ca^{++}- or Na^{+}-free solution before the application of TTX or verapamil.

* Significantly different from MTX alone in each solution, $P < .01$.

Finally, scaritoxin, a hydrohobic compound isolated from a ciguatera fish *Scarus gibbus,* has a releasing action of NE and ACh from adrenergic and cholinergic nerve endings, resulting in the contraction of the guinea-pig vas deferens and ileum, respectively (Tatsumi *et al.,* 1985).

ACKNOWLEDGMENTS

I am greatly indebted to Prof. T. Yasumoto of Tohoku University for his generous supply of MTX and to Prof. J. Scheuer and Dr. K. Tachibana of the University of Hawaii for providing CTX.

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